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Docket No.: C03123/119364

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of :
John DAPRON *et al.*

Serial No.: 09/854,838

Filed: May 14, 2001

For: HIGH CAPACITY ASSAY PLATFORMS

Examiner: My Chau T. TRAN

Art Unit: 1641

Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF DR. WILLIAM KAPPEL UNDER 37 CFR 1.132

I, William Kappel, a citizen and a resident of the U.S., hereby declare as follows:

1. I received a B.S. Degree in Biological Science from Oregon State University in 1971. I received a Ph.D. degree in Biochemistry from The Pennsylvania State University in 1976. My Ph.D. thesis was entitled "The Isolation and Partial Characterization of Diphosphoglycerate Mutase From Human Erythrocytes." From 1976-1979, I was a post-doctoral fellow in the Department of Biochemistry and Biophysics at the University of California Davis. As a post-doctoral fellow, I investigated the structure-function relationship of the pyridoxal-5'-phosphate allosteric regulator binding site of *E. coli* ADP-glucose pyrophosphorylase.

2. From 1979-1982, I was an Assistant Research Professor in the Department of Biochemistry at the St. Louis University School of Medicine. From 1982-

1985, I was an Assistant Professor in the Department of Biochemistry at the University of Pittsburgh School of Medicine.

3. Since 1985, I have been continuously employed by Sigma Chemical Company or an entity owned by Sigma-Aldrich Corp. I am currently employed by Sigma-Aldrich Biotechnology as R&D Manager in the Protein R&D Department. My current responsibilities include directing Sigma's research and development efforts for new products in the proteomics field, including efforts to improve and develop high-throughput screening platforms.

4. The present application discloses and claims an assay platform that comprises a substrate and a polymer matrix attached to the substrate. The polymer matrix is capable of binding target molecules and comprises a plurality of polymer molecules. At least some of the polymer molecules are covalently attached directly to the substrate, some of the polymer molecules are crosslinked to other polymer molecules and some of the polymer molecules have at least one binding ligand covalently attached thereto. The density of the polymer matrix on the substrate is at least 2 $\mu g/cm^2$. (Specification, p. 2, ¶0006; p. 3, ¶0010 – p. 6, ¶0018; Examples 1, 2 and 4-7; and claim 1).

5. In the above-identified application, it has been demonstrated that the presently claimed assay platforms provide a high density polymer matrix attached to a substrate that has the appropriate architecture for binding large quantities of proteins and other molecular components. (Abstract; Specification, pp. 3-4, ¶0010). This architecture is achieved and may be further tailored by the newly identified ability to provide polymer matrix densities of at least 2 $\mu g/cm^2$ on a suitable substrate. (*See e.g.*,

specification, pp. 4-5, ¶0010; p. 6, ¶¶0017-0018; and p. 8, ¶26). Based on these observations, a significant advance is achieved by proceeding in accordance with the presently claimed assay platforms for isolation of multi-microgram amounts of proteins and other molecular components for use in e.g., high throughput, multi-sample screening. (Specification, pp. 4-5, ¶0010 and Fig. 1).

6. I am aware that an Office Action has issued with regard to the present application. It is my understanding that in the Office Action, the Examiner asserted that the claimed assay platform is not novel or patentably distinct from certain prior art documents. The Examiner has relied on a single prior art document, Bioprobe International, Inc., International Publication No. WO 92/03732 ("Bioprobe"), as disclosing an assay platform containing a coating material having a number of functional binding sites available for binding of ligands that may be used in assay methods involving solid phase materials. (Paper No. 5 at 5-7). Thus, the linchpin of the Examiner's position, which runs through all of the prior art rejections, is that because Bioprobe discloses a polymer matrix that contains all of the features recited in, e.g., claim 1, namely "that the polymer binds to the substrate, that the polymers are crosslinked to other polymers and attached to a ligand," therefore the density of the polymer disclosed in Bioprobe would "inherently" be at least 2 $\mu g/cm^2$. (*Id.* at 5).

7. I am familiar with Bioprobe cited in the pending Office Action (Paper No. 5 at 5-7). Bioprobe discloses compositions and methods for preparation of assay systems, in particular, "solid phase" systems. (p. 1, lns. 7-8). Bioprobe discloses "water-soluble compounds (both monomers and polymers) including hydrophobic moieties that bind tightly to, e.g., the plastics commonly used as solid phases ...." (p. 3,

ln. 33 - p. 4, ln. 1). These compounds "further carry reactive functional groups (*e.g.*, hydrazide or 2-(N-methylpyridyl) groups) which form stable covalent bonds with ligands ...." (p. 4, lns. 1-4). Bioprobe discloses a general formula for such reagents/coating materials as:

$$(R^N\text{-})_x A (\text{-}X)_y$$

wherein

R^N represents a hydrophobic moiety which develops substantial nonspecific interactions with a hydrophobic material, such as solid phase materials;

A is an organic spacer group;

X represents a reactive group; and

x, y represent an integer from 1 to about 1300. (*Id.* at lns. 8-21).

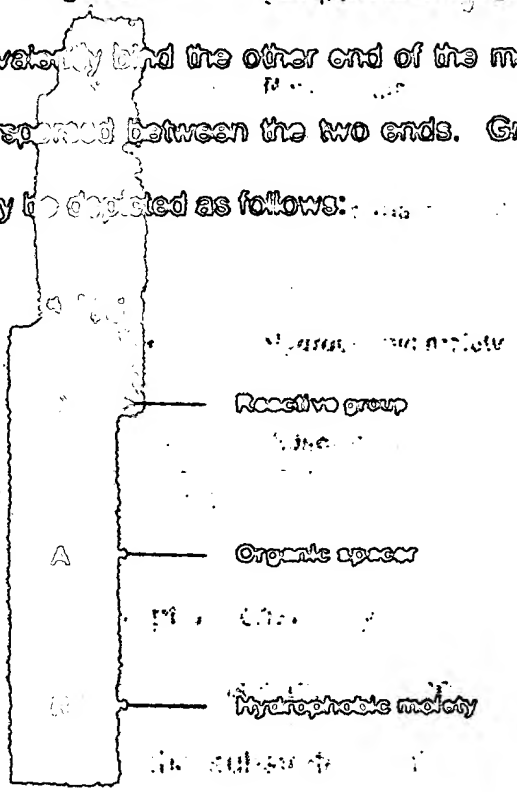
Bioprobe further discloses methods for avoiding crosslinking of the polymeric materials. For example, Bioprobe discloses that "[w]hereas the direct reaction of a reactive dihydrazide (*e.g.* adipic dihydrazide) tends to result in the formation of insoluble products (probably due to crosslinking), the addition of small amounts of hydrophobic hydrazides to the dihydrazide reaction mixture tends to inhibit crosslinking, permitting a longer reaction time and a more complete reaction." (p. 10, lns. 14-21). In all of the Bioprobe examples, to prepare the surface of a solid phase for binding to ligands, the reagent/coating material was applied to the solid phase, optionally incubated for a period of time and then washed off. (p. 12, ln. 3 – p. 32, ln. 18). Table 2 shows resistance to detergent (*i.e.*, Tween-20) of two different coating materials applied to

polystyrene microtiter plates. (p. 20, lns. 1-15). For the dextran-hydrazide and dextran-phenylhydrazide coating materials, at 10% Tween-20, 26.91% and 66.74% binding of rabbit anti-HRP was disclosed, respectively. (*Id.*).

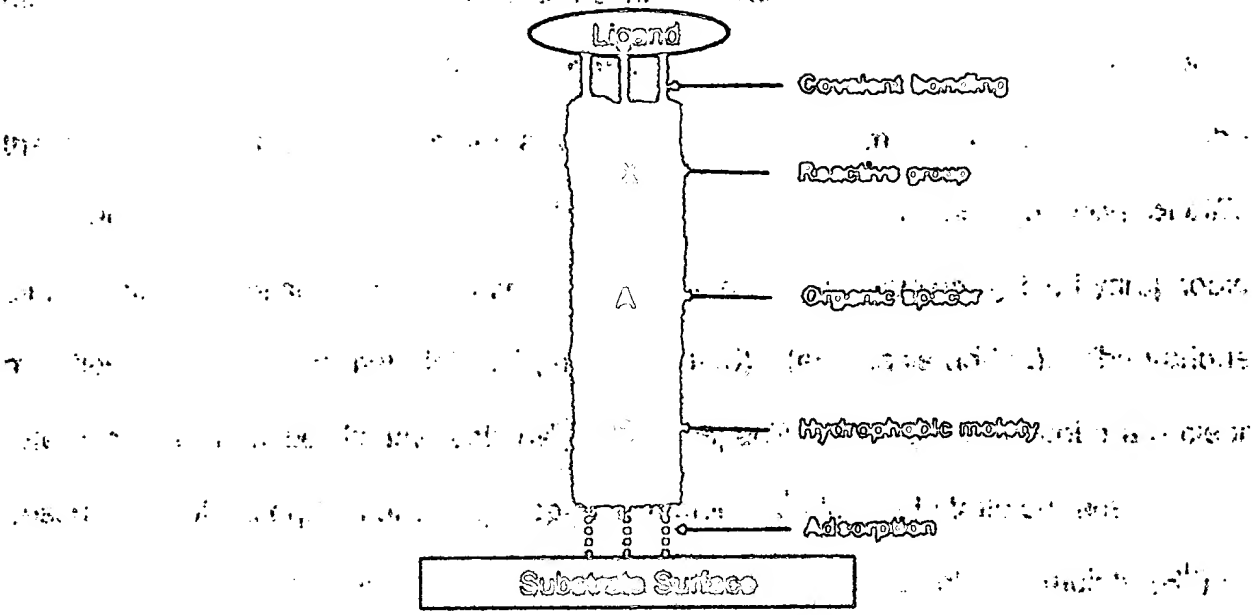
8. I am aware of no published document that describes or even suggests that it was possible to achieve polymer matrix densities anywhere approaching the "at least 2 $\mu g/cm^2$" recited in the present claims using conventional adsorption chemistry of the kind described in Bioprobe. Thus, I was surprised to note the Examiner's conclusion that Bioprobe "inherently" discloses a polymer density of "at least 2 $\mu g/cm^2$."

9. To demonstrate that Bioprobe does not disclose, inherently or otherwise, a density of its reagent/coating material on a substrate of "at least 2 $\mu g/cm^2$" as required by the present claims, I have reviewed the published literature in this area with a view toward determining the maximum achievable density of a Bioprobe reagent/coating material on a substrate using the processes disclosed in Bioprobe. As set forth in more detail below, I have found that using the Bioprobe methodology and materials, the Bioprobe reagent/coating material forms, at best, a monolayer over the surface of a substrate. As such, the literature demonstrates that the theoretical maximum density of a polymer achievable for a monolayer coating on a plastic substrate is about 300 ng/cm^2. This density is more than six-fold less than the minimum density required by the present claims. Based on these results, it is clear to me that Bioprobe does not disclose, inherently or otherwise, a critical element recited in the present claims. Nor does Bioprobe suggest the "at least 2 $\mu g/cm^2$" requirement of the present claims.

10. Bioprobe discloses a bipartite reagent, "$(R^H\text{-})_n$ A $(\text{-}X)_m$," designed to adsorb one end (R^H) of the reagent/coating material to a substrate through hydrophobic (non-covalent) interactions and to covalently bind the other end of the molecule (X) to ligands with a spacer group (A) interposed between the two ends. Graphically, the Bioprobe reagent/coating material may be depicted as follows:



11. In a preferred embodiment, Bioprobe asserts that because the disclosed polymers "are designed to bind to the solid phase by hydrophobic interactions, hydrazide groups may be placed on a variety of plastics quickly and easily by adsorption." (Bioprobe, p. 10, ln. 33 – p. 11, ln. 2). Every example in Bioprobe is consistent with the general description of the reagent/coating material and the exemplified "preferred embodiment" in that every exemplified reagent/coating material was adsorbed onto the surface of a solid substrate (*e.g.*, bead, plastic plate) through the hydrophobic moiety (R^H). Graphically, the Bioprobe reagent/coating material adsorbed onto a surface of a substrate and covalently bonded to a ligand may be depicted as follows:



12. A basic principle in adsorption chemistry, *i.e.*, the chemistry of passively attaching molecules to the surface of a substrate, is the characteristic pattern of monolayer deposition of the molecules on the substrate. This is because of the nature of the forces that anchor the molecules to the substrate. These forces are called intermolecular attraction forces. These forces are based on intramolecular electric polarities and are to be distinguished from covalent bonding (*i.e.*, sharing of electrons).

13. It is my understanding that the Bioprobe reagent/coating material is attached to the surface of a substrate through adsorption, *i.e.*, through intramolecular attraction forces. My understanding is based upon specific disclosure in Bioprobe, as well as my knowledge and experience in this field. Bioprobe, for example, provides no description of covalently bonding R^H to a substrate surface, but does disclose that R^H "develops substantial *nonspecific* interactions with a hydrophobic material, such as a solid substrate" (Bioprobe, p. 4, lns. 11-14); "[t]he desired coating material is provided in a hybrid form as a combination of (1) one or more hydrophobic moieties designed to

maximize the *nonspecific* binding of the coating material with the solid phase materials ..." (*Id.*, p. 7, lns. 1-5); "any effects of *nonspecific* (*e.g.*, hydrophobic) interactions on the properties of the coating material with the solid phase minimize or eliminated the nonspecific binding of ligands to the solid phase." (*Id.*, lns. 9-13); and "[t]he *nonspecific* binding sites of the solid phase materials are preferentially occupied by the hydrophobic moieties of the coating material, ..." (*Id.*, lns. 13-15). (emphasis added). The various references to "nonspecific interactions" and "nonspecific binding" in Bioprobe are clear descriptions of adsorption of the hydrophobic moiety (R^H) to a substrate surface.

14. It is further my understanding that the hydrophobic moiety (R^H) of Bioprobe is dispersed on the substrate as a monolayer or as an incomplete monolayer. My understanding is based on the description in Bioprobe that the "optimal coating concentrations" for dextran-adipic hydrazide and dextran-adipic hydrazide with hydrophobic groups was 200 µg/ml and 100 µg/ml, respectively and that at "high coating concentrations" binding of oxidized IgG is reduced. (p. 15, lns. 9-22 and Fig. 1).

15. It is well documented in the literature that polymeric moieties containing hydrophobic segments, *e.g.*, polypeptides or polymers, may be passively adsorbed, as a monolayer, to a hydrophobic surface such as polystyrene, only up to about 300 ng/cm^2, although this estimate may vary insubstantially based on the dimensions of the specific moiety used (*e.g.*, for a lense-shaped IgG molecule, the density is estimated to be between 130-650 ng/cm^2). The maximum density on a substrate (Q) for an adsorbed polymer moiety is calculated (assuming maximally/efficiently adsorbing a polymer moiety onto a substrate surface) according to the following formula:

$$Q = \frac{2}{\sqrt{3}} \cdot \frac{MW}{N} \cdot \frac{1}{(2r)^2} \cdot 10^9 \, ng/cm^2$$

where:

MW = molecular weight

N = Avogadro's number

r = Stokes radius of polymer moiety = $\frac{R \cdot T_{20}}{6 \cdot \Pi \cdot \eta_{20} \cdot D_{20} \cdot N}$ cm

R = gas constant

T_{20} = room temperature

η_{20} = viscosity of water at 20°C

D_{20} = differential coefficient of polymer moiety to water at 20°C.

The maximum density, Q, will not change considerably within wide molecular weight limits. *See e.g.*, Deshpande, S.S., Enzyme Immunoassays From Concept To Product Development. 193-228, Chapman & Hall (1996) and NUNC™ Bulletin No. 6(1) Principles In Adsorption To Polystyrene 2nd Ed. (1997) copies of which are attached as Exhibits 1 and 2. Thus, it is appropriate to use the 300 ng/cm^2 value for the Bioprobe reagent/coating material.

16. Based on the adsorption kinetics described throughout Bioprobe and exemplified in Fig. 1, the optimal concentration ranges of 100-200 µg/ml identified, the reduction in IgG binding at high coating concentrations of hydrazide polymers and the theoretical maximum polymer density on a substrate in ¶15, it is my understanding that, at best, using the Bioprobe materials and methods, a monolayer could be formed on the substrate surface and that the density of the Bioprobe reagent/coating material on such a surface would be about 300 ng/cm^2.

17. All of the rejected claims, however, require that the density of the polymer matrix on the substrate be "at least 2 $\mu g/cm^2$." See e.g., claim 1. Such a density *cannot* be achieved with adsorption and is only achievable through covalent attachment of some of the polymer 3D matrix molecules to the substrate. Thus, all of the rejected claims require a minimum density of the polymer matrix on the substrate that is over six-fold higher than the maximum amount that is described in Bioprobe.

18. Based on my knowledge and experience, and in view of the results presented herein, it is my opinion that Bioprobe does not disclose, inherently or otherwise, a density of the reagent/coating material on the substrate of "at least 2 $\mu g/cm^2$" and, in fact, discloses the coating of a substrate in monolayer or sub-monolayer densities. Furthermore, in view of Bioprobe's clear teachings that the hydrophobic moiety (R^H) is adsorbed onto the surface of the substrate, that high concentrations of the reagent/coating material cause reduced IgG binding and that inhibiting crosslinking permits longer and more complete reaction of the reagent, Bioprobe would not have suggested or provided a motivation to use a higher polymer density, for example a density of the polymer matrix on the substrate of "at least 2 $\mu g/cm^2$" as required by the presently rejected claims. Moreover, in view of Bioprobe's disclosure that using high concentrations of the reagent/coating material to coat a substrate results in a reduction of binding of oxidized IgG, it would have been unexpected to observe the superior binding capacity achieved using the assay platform of the present invention wherein the density of the polymer matrix is at least six-fold greater than the density achievable using the Bioprobe reagent/coating material and methods.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: Jan 27, 2003

William Kappel

William Kappel, Ph.D.